CVI. THE EFFECT OF INANITION AND VITAMIN B DEFICIENCY ON THE ADRENAL GLANDS OF THE PIGEON.

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INTRODUCTION.

IN a recent communication [Marrian *et al.*, 1927] it was shown that the hypertrophy of the adrenal glands which can be produced in pigeons by allowing them to feed voluntarily on a diet deficient in vitamin B, could also be produced by inanition even when liberal supplies of extracts rich in the vitamin were fed. It was at first thought that this indicated that the adrenal hypertrophy of vitamin B deficiency was entirely due to the state of inanition that almost invariably accompanies this condition. However, they found that when the pigeons were forcibly fed with the deficient diet, so that the loss of body weight at the conclusion of the experiment was negligible, the adrenals were enlarged to approximately the same extent.

The present work was undertaken in order to confirm and if possible find some explanation for these curious results. In addition it was thought desirable to investigate the possibility of a deficiency of one only of the two recently recognised factors in the vitamin B complex being involved in the causation of this hypertrophy.

EXPERIMENTAL.

The observations reported here were carried out on the same series of pigeons upon which a study of the effect of vitamin B deficiency and inanition on the testis was carried out [Marrian and Parkes, 1928].

Male birds were exclusively used, since the size of the adrenal glands seems to vary appreciably with sex, and in the case of the hen bird there would appear to be a definite hypertrophy during ovulation [Riddle, 1922].

The birds were then divided into five groups which were arranged and treated in the following manner:

Group	Diet	Vitamin preparations given	Object of experiment
A	20 g. vitamin B-deficient diet daily forcibly fed	1 g. yeast extract daily	Normal controls
B	" "	—	Vitamin B deficiency
C	" "	1 g. autoclaved yeast extract daily	Vitamin B ₁ deficiency
H	" "	Peters's torulin curative dose × 3	Vitamin B ₂ deficiency
SM	Starving	1 g. yeast extract daily	Inanition

The deficient diet used was an artificial one, of the type used by Randoin and Simmonet [1924]. The method of its preparation and the technique of forcible feeding have been described fully elsewhere [Kon and Drummond, 1927; Marrian *et al.*, 1927].

The vitamin preparations were administered to the birds in aqueous solution directly into the crop through a rubber catheter tube. The autoclaved yeast extract (known to contain no vitamin B₁) was prepared by heating a slightly alkaline solution of commercial yeast extract to 110° for 3 hours. Before use this was carefully neutralised. The preparation containing vitamin B₁ only was prepared according to the method of Kinnersley and Peters [1927].

In order to avoid errors in body weight due to overloaded crops, the birds were all starved for 24 hours before the initial weighings were made. At the conclusion of the experiments any food remaining in the crop was quantitatively removed, dried and weighed. The final weight of each bird was obtained by subtracting this figure from the total body weight immediately before death.

The vitamin B₁-deficient pigeons (groups *B* and *C*) were killed at the first signs of typical polyneuritis, while those in groups *A* and *H*, which would not be expected to show terminal symptoms were killed as soon after as possible. The starving pigeons were killed at the beginning of the final collapse, which has been described by Marrian *et al.* [1927].

Immediately after the death of the bird the adrenal glands were cut out, carefully dissected from any adhering connective tissue and fat, and weighed. Adrenaline was estimated in some of these by the modified Folin, Cannon and Denis method suggested by Baker and Marrian [1927]. Estimations of the moisture content of a number of others were carried out to investigate the possibility of the observed hypertrophy being to some extent due to oedema.

General behaviour of the birds.

A brief description only of the general behaviour of this series of pigeons will be made, since this has been dealt with elsewhere [Marrian and Parkes, 1928].

With one exception, which will be referred to in a later section of the paper, these birds behaved in a normal manner and in all cases the body weights were satisfactorily maintained. The majority of the birds in groups *B* and *C* showed the classical symptoms of neck retraction, but in a few instances the condition called *emprosthotonus* by McCarrison [1919] was observed. The group *H* birds, like those in groups *B* and *C*, vomited daily to some extent, but otherwise no definite symptoms could be observed.

Since the body weights of nearly all the birds in these groups were satisfactorily maintained, it is evident that the greater part of the diet was retained and satisfactorily metabolised.

The general condition of the pigeon during inanition has been fully discussed previously [Marrian *et al.*, 1927].

Details of the weight changes, symptoms, and times on the diets of the birds are shown in Table I.

Weight of the adrenal glands.

On referring to Table I it will be seen that the adrenals of the pigeons that were forcibly fed on the deficient diet and also those of the starving birds receiving vitamin B are definitely hypertrophied when compared with those of the forcibly fed controls in group *A*. This confirms the findings of Marrian *et al.* [1927].

The results from the birds in groups *C* and *H*, deficient in vitamins B₁ and B₂ respectively, are however not quite so clear cut. The average figure for the weight of the glands of the group *C* birds seems to indicate that the hypertrophy is of almost the same order as in group *B*. The weight of the adrenals of *C* 66 however was more than twice the average of the remainder in the group, and since this bird was abnormally oedematous it would almost seem justifiable to eliminate this result. If this is done, an average figure for the adrenal weight in group *C* of 0.0425 g. is obtained, which, although quite definitely greater than the control, is considerably less than the group *B* average.

The average weight of the adrenals of the group *H* pigeons is quite clearly significantly less than the group *B* average, but still definitely above that of the control group. The possibility must be borne in mind that these birds were vomiting an appreciable quantity of the antineuritic vitamin and that in consequence they were suffering from a mild vitamin B₁ deficiency. This however seems unlikely since the birds were receiving three times the normal curative dose daily by mouth and in addition were injected with the extract on three occasions.

The author is inclined to the view that this slight adrenal hypertrophy of the pigeons deficient in the thermostable factor is quite definite and also that the difference in degree of hypertrophy between groups *B* and *C* is real and significant. Thus it would appear that a deficiency of each of the two factors plays a part in the production of the adrenal enlargement observed in vitamin B deficiency, the vitamin B₁ deficiency being the more important.

This conclusion is in agreement with the recent observations of Findlay [1928] who has shown that the adrenal glands of rats are enlarged as a result of vitamin B₁ or B₂ deficiency. The greater degree of hypertrophy of the adrenals of Findlay's rats deficient in vitamin B₂ only, when compared with the group *H* pigeons, may be explained by the fact that they were fed on the deficient diet for over twice as long as those deficient in vitamin B₁, whereas the pigeons in groups *C* and *H* were receiving the deficient diets for approximately the same length of time.

Another possible explanation of these results is suggested by the recent

work of Williams and Waterman [1927] who on rather scanty evidence suggest that a third factor (present in whole wheat) is necessary for the satisfactory health and growth of the pigeon, vitamin B₂ being relatively unimportant. Assuming that it is present in yeast extract, it is conceivable that the adrenal hypertrophy might in some degree be due to a deficiency in this factor.

Table I.

Group	No.	Initial weight (g.)	Final weight (g.)	Weight change %	Days on diet	Weight adrenals (g.)	Total adrenaline (mg.)	Adrenaline per 1 g. gland (mg.)	Moisture content of adrenals %	Remarks on condition of bird when killed
A	5	405	475	+19.7	18	0.0232	0.057	2.46	—	—
A	3	389	476	+22.4	25	0.0270	0.068	2.52	—	—
A	6	292	348	+19.1	26	0.0277	0.062	2.24	—	—
A	39	446	500	+12.1	22	0.0174	—	—	70.7	—
A	43	406	452	+11.3	22	0.0287	0.060	2.09	—	—
A	42	471	475	+ 0.8	23	0.0352	—	—	71.3	—
A	40	434	443	+ 2.1	24	0.0294	—	—	74.7	—
A	38	421	440	+ 4.3	25	0.0268	—	—	72.0	—
A	44	346	420	+17.6	25	0.0248	—	—	70.5	—
A	37	411	390	- 5.1	26	0.0297	—	—	73.3	—
A Average		402	442	+10.4	24	0.0270	0.062	2.33	72.1	—
B	7	394	398	+ 1.0	17	0.0539	0.066	1.22	—	Severe neck retraction
B	9	382	352	- 7.8	18	0.0813	0.172	2.12	—	Neck retraction
B	8	401	434	*	18	0.0696	0.078	1.12	—	Neck retraction. Breast very oedematous
B	11	350	354	+ 1.1	21	0.0810	0.138	1.17	—	Emprothotonus
B	10	343	279	-18.6	22	0.0515	0.063	1.22	—	Neck retraction
B	52	423	411	- 2.8	16	0.0586	—	—	75.2	Weak. Slight neck retraction
B	45	406	393	- 3.2	17	0.0573	—	—	76.1	Severe neck retraction
B	51	516	456	-11.6	19	0.1062	—	—	75.9	Neck retraction
B	50	431	355	-17.6	19	0.1023	—	—	75.6	Emprothotonus
B	48	424	350	-17.4	24	0.0424	—	—	75.5	Severe neck retraction
B Average		407	378	- 7.7	19	0.0704	0.103	1.37	75.7	—
C	18	394	386	- 2.0	22	0.0376	0.051	1.36	—	Neck retraction
C	57	348	368	+ 5.7	18	0.0441	0.045	1.02	—	Emprothotonus
C	59	439	444	+ 1.0	22	0.0677	0.051	0.74	—	Emprothotonus
C	66	427	519	*	15	0.1222	0.122	1.00	—	Neck retraction. Breast extremely oedematous. Abdominal cavity full of fluid
C	61	320	325	+ 1.6	16	0.0294	0.038	1.29	—	Emprothotonus
C	64	435	481	+10.5	18	0.0399	0.102	2.55	—	Severe neck retraction
C	65	430	419	- 2.5	20	0.0360	0.062	1.72	—	Neck retraction
C Average		399	420	+ 2.4	19	0.0538†	0.067	1.38	—	—
H	70	372	324	-12.8	23	0.0508	0.084	1.65	—	—
H	68	395	407	+ 2.9	23	0.0282	0.063	2.23	—	—
H	69	345	320	- 7.2	23	0.0330	0.073	2.21	—	—
H	72	441	374	-15.0	23	0.0345	0.105	3.03	—	—
H	67	371	336	- 9.4	23	0.0343	0.091	2.65	—	—
H Average		385	352	- 8.3	23	0.0361	0.083	2.35	—	—
SM	75	312	204	-34.6	11	0.0358	0.088	2.46	—	—
SM	73	324	202	-37.7	17	0.0526	0.122	2.32	—	—
SM	74	387	237	-38.8	17	0.0533	—	—	79.2	—
SM	76	435	273	-37.2	21	0.0523	—	—	77.2	—
SM	78	478	285	-40.4	22	0.0528	0.137	2.59	—	—
SM Average		387	240	-37.7	18	0.0494	0.116	2.46	78.2	—

* Both these birds were extremely oedematous, hence the body weight changes are of little significance.

† 0.0425, excluding pigeon C 66.

Oedema of the adrenal glands.

A number of determinations of the moisture content of the adrenals of the groups *A*, *B* and *SM* birds were carried out in order to see to what extent the adrenal enlargement in the two latter groups could be accounted for by an increase in the water content of the glands. Table I clearly shows that in both cases the glands were quite distinctly oedematous.

In the case of the starving birds this oedema was so marked as to account for nearly half of the total observed hypertrophy.

The following table summarises these results.

Table II.

Group	Fresh weight adrenals g.	Dry weight adrenals g.	Water %	Hypertrophy calculated on fresh weight %	Hypertrophy calculated on dry weight %	Proportion of hypertrophy due to oedema %
<i>A</i> (av. of 6)	0.0272	0.0075	72.1	—	—	—
<i>B</i> (av. of 5)	0.0734	0.0178	75.7	169	137	19
<i>SM</i> (av. of 2)	0.0528	0.0115	78.2	94	53	44

The nature of the adrenal hypertrophy.

Several observers seem to be agreed that during inanition there is an increase in adrenaline roughly corresponding to the enlargement of the gland in the pigeon [McCarrison, 1919, 1920; Marrian *et al.*, 1927]. The three adrenaline estimations carried out on the *SM* group of pigeons seem to confirm these observations, although the experiments are admittedly too few in number. So far as the author is aware, no histological study has yet been made of the adrenal glands of starving pigeons, so that it is as yet impossible to say definitely whether this represents an actual proliferation of the medullary cells, a hyperactivity of the medulla which remains constant in size, or a decreased output of adrenaline. As a result of studies carried out on pigeons fed on polished rice, Kellaway [1921] explains the increased adrenaline content of the hypertrophied glands by postulating that the adrenaline output is decreased by reason of the lowered metabolism of the bird. This however seems to be unlikely since at the time when the enlarged gland shows a high adrenaline content, the cloacal temperature of the bird is not necessarily markedly subnormal. In the case of the adult rat Vincent and Hollenberg [1921] showed that the adrenaline content of the adrenals is increased after 2–3 days' starvation, and at this early stage of inanition the body temperature and metabolism of the rat are not greatly reduced [Drummond and Marrian, 1926]. McCarrison [1920] regards the increased adrenaline content of the glands as representing true medullary hypertrophy. This is regarded as being the more acceptable hypothesis.

A discussion of the nature of the hypertrophy resulting from vitamin B deficiency is rendered difficult owing to the facts that many of the birds

examined by previous workers have been virtually starving at the time of incidence of the nervous symptoms and that much of the evidence is apparently conflicting. Thus McCarrison [1919] examined starving pigeons that had lost an average of 23 % of their body weight, while his polished rice birds had lost about 30 %. It is not surprising therefore that these birds gave a similar series of results to his starving ones. Kellaway [1921] made a study of pigeons fed voluntarily and forcibly on polished rice. In both cases he showed that there was a marked increase in the adrenaline content of the glands. His forcibly fed birds, however, although showing a loss of body weight (15 %) were far from being in a state of inanition. Beznak [1923] on the other hand, could find no increased amount of adrenaline in the hypertrophied glands of polyneuritic pigeons that had lost as much as 22 % of their original body weight.

The histological evidence suggests that it is the cortex that is mainly involved in the hypertrophy [Findlay, 1921; Lasowsky and Sumnitzki, 1926]. Findlay indeed showed that after curing polyneuritic pigeons with yeast, there occurred a rapid decrease in the cortical lipoids. It seems fairly clear therefore that in experimental beriberi changes do occur in the adrenal cortex.

Marrian *et al.* [1927] showed that in five forcibly fed vitamin B-deficient pigeons, whose average loss of body weight was only 5 %, the adrenals were considerably hypertrophied while the adrenaline content was not increased. It was pointed out that this might be explained on the assumption that the cortex alone hypertrophied when starvation did not complicate the picture.

However in the five group B pigeons that were examined, two cases (B 9, B 11) were found where the adrenals contained an abnormally large amount of adrenaline. The other three cases were entirely in line with the results of Marrian, Baker, Drummond and Woollard. It seems to be necessary therefore to conclude that in certain cases of experimental beriberi where the adrenaline content of the glands is increased, even when the starvation factor is eliminated, the medulla may be involved in the hypertrophy to some extent.

In spite of the somewhat disturbing nature of these results the bulk of the evidence certainly seems to indicate that the cortex is the site of the adrenal hypertrophy in most cases of vitamin B deficiency, the medulla being involved when starvation complicates the picture and also in certain other random cases.

This hypothesis receives some support from recent work on the function of the adrenal cortex. Evidence based on observations on adrenalectomised animals is accumulating to show that the adrenal cortex has the function of removing or neutralising in some manner various toxins, bacterial or otherwise, in the body [Lewis, 1923; Jaffe, 1926; Banting and Gairns, 1926; Belding and Wyman, 1926; Torino and Lewis, 1927], although this theory is strenuously denied by Stewart, Rogoff and their collaborators in a series of papers.

It also seems to be an established fact that bacterial infections and certain types of intoxication may cause an enlargement of the adrenal cortex.

It is interesting to note that McCarrison [1919] showed that in polyneuritic pigeons that were also suffering from various bacterial infections the adrenals were considerably enlarged without any corresponding increase in the amount of adrenaline. Since the symptoms in such birds were observed at an earlier date than in uninfected ones, one might expect that the starvation factor would be decreased or altogether eliminated. Thus the hypertrophy might be due either to vitamin B deficiency, or to the bacterial toxin, or more probably to both.

Origin of the nervous symptoms arising from vitamin B deficiency.

The view has often been expressed that the underlying cause of the nervous symptoms observed in vitamin B deficiency is a toxic substance. Thus one might build up an attractive theory explaining the adrenal hypertrophy in beriberi as an attempt by the body to eliminate the "beriberi" toxin. It should be possible to test this theory by showing that adrenalectomised animals develop beriberi more rapidly than do normal animals. Several unsuccessful attempts have been made by the author to do this. A few initial experiments were carried out on rats and in one instance convulsive seizures were observed a few days after the animal had been placed on the deficient diet. There were no grounds to believe however that these convulsions were specifically due to the vitamin deficiency, since convulsions are often a symptom of adrenal insufficiency in normally fed animals. In addition it has been shown by the author that adrenalectomised rats survive starvation for a much smaller period than normal rats (unpublished work), and since marked loss of appetite is a symptom both of adrenal insufficiency and vitamin B deficiency, it seemed to be useless to continue such experiments.

Beriberi-like symptoms in normal pigeons.

An extraordinary case was observed among the group *A* pigeons, which were receiving 1 g. of yeast extract daily. On the 19th and 20th days it was noticed that pigeon *A* 4 was far from well. After each meal there was considerable vomiting and in general it may be said that the condition of the bird closely resembled that of the vitamin B-deficient pigeons just before the incidence of the nervous symptoms. On the morning of the 21st day this bird was found in a condition absolutely indistinguishable from the usual symptoms of experimental "beriberi." The head of the bird was severely and persistently retracted and there was a periodic tendency to turn "cart-wheels." It would be no exaggeration to say that this bird seemed to be a more characteristic case of "beriberi" than many of those fed on deficient diets. The initial weight of the pigeon was 389 g. At the time that the symptoms appeared the total body weight was 433 g. The weight of food in

the crop was judged to be no more than 30 g., so evidently no loss of body weight had occurred.

It appeared extremely unlikely that this curious condition could be caused by a vitamin deficiency resulting from the vomiting of the administered yeast extract since no vomiting had been noticed until the 19th day. Even if the whole of the yeast extract had been expelled on the 19th and 20th days, it is quite inconceivable that this short period of deficiency could be the cause of the symptoms.

After some hours, as the condition remained unchanged, it was decided to see if the condition was curable by an injection of antineuritic vitamin. Accordingly 1 cc. of an extract made by the method of Kinnersley and Peters, that was known to be curative in doses of 0.5 cc., was injected. After 3 hours the pigeon appeared to be no better. The following morning the symptoms were still severe although the bird appeared considerably weaker. It was quite evident therefore that this condition was not curable by vitamin B₁. The bird was subsequently killed while still showing these symptoms.

This case goes to strengthen the belief, which is rapidly growing among workers on beriberi in the pigeon, that the symptom of neck retraction is not specific to vitamin B deficiency and in fact may occur as a result of a variety of causes. Thus Marrian *et al.* [1927] observed symptoms of neck retraction in starving pigeons that were receiving ample supplies of yeast extract, and Williams [1927] reports a similar condition in pigeons that were fed exclusively on rice polishings. The author has also observed on one occasion well-defined neck retraction in a normal pigeon that was partially anaesthetised with ether.

SUMMARY.

1. The results obtained by Marrian, Baker, Drummond and Woollard showing that adrenal hypertrophy occurred in starving pigeons receiving vitamin B and also in pigeons forcibly fed on an artificial vitamin B-free diet to exclude the starvation factor, have been confirmed.

2. The hypertrophy occurring in the vitamin B-deficient pigeons seems to be mainly due to a deficiency in vitamin B₁, but it is considered that a deficiency of vitamin B₂ is also a contributory factor.

3. The adrenal glands of both the starving and the vitamin B-deficient pigeons are oedematous. This oedema accounts for 19 % of the total hypertrophy shown by the vitamin B-deficient birds and 44 % in the case of the starving pigeons.

4. The question whether the adrenal hypertrophy occurring in starvation and vitamin B deficiency is medullary or cortical is discussed. It is considered possible that in starvation the changes occur mainly in the medulla while in vitamin B deficiency the cortex is mainly concerned. In certain cases changes in the medulla may occur in vitamin B deficiency.

5. A brief reference to a pigeon, forcibly fed on a complete diet, that developed acute symptoms resembling those due to vitamin B deficiency is made. This condition, although identical in appearance with avian beriberi, was not curable by vitamin B₁.

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